Evidence for the Involvement of Dopamine Transporters in Behavioral Stimulant Effects of Modafinil

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ABSTRACT

Modafinil is prescribed for numerous medical conditions, but the drug's mechanism of action is unclear. Here, we examined the interaction of modafinil with receptors and transporters in vitro and compared pharmacological effects of the drug with those produced by indirect dopamine (DA) agonists 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR12909) and (+)-methamphetamine (METH). Modafinil was screened at various receptors and transporters using binding assays. Transporter-mediated uptake and release were examined in rat brain synaptosomes. Effects of modafinil on motor activity and neurochemistry were determined in rats undergoing in vivo microdialysis in nucleus accumbens. Of the receptors and transporters assayed, modafinil displayed measurable potency only at DA transporters (DAT), inhibiting [3H]DA uptake, with an IC_{50} value of 4.0 μM. Accordingly, modafinil pretreatment (10 μM) antagonized METH-induced release of the DAT substrate [3H]1-methyl-4-phenylpyridinium. Intravenous modafinil (20 and 60 mg/kg) produced dose-dependent increases in motor activity and extracellular DA, without affecting serotonin (5-HT). Analogous results were observed for GBR12909 (1 and 3 mg/kg), whereas METH (0.3 and 1 mg/kg) increased DA and 5-HT. Locomotor effects of all drugs were positively correlated with dialysate DA ($P < 0.001$). Interestingly, modafinil pretreatment reduced METH-induced ambulation and DA release. Our data show that modafinil interacts with DAT sites in rat brain, a property shared with agonist medications under investigation for treating cocaine dependence. Nondopaminergic mechanisms may also contribute to the pharmacology of modafinil. Finally, the results suggest that modafinil should be tested as an adjunct for treating METH addiction.

Modafinil (2-[(diphenylmethyl) sulfinyl] acetamide) is a wake-promoting agent approved for the treatment of narcolepsy (Wise et al., 2007). Recently, modafinil has been prescribed for other psychiatric disorders such as attention-deficit hyperactivity disorder (Swanson et al., 2006) and cocaine dependence (Dackis et al., 2005). In a clinical laboratory setting, modafinil pretreatment reduces cocaine self-administration (Hart et al., 2008) and positive subjective effects (Dackis et al., 2003; Malcolm et al., 2006), supporting the utility of the drug as a pharmacotherapy for stimulant addiction. The off-label use of modafinil for treating cocaine dependence is noteworthy because no approved medications

ABBREVIATIONS: DA, dopamine; 5-HT, 5-hydroxytryptamine (serotonin); DAT, dopamine transporter(s); NE, norepinephrine; NET, norepinephrine transporter(s); GBR12935, 1-[2-benzhydroxyethyl]-4-(3-phenylpropyl)piperazine; METH, (+)-methamphetamine; RTI-55, 3β-(4-iodophenyl)-tropan-2β-carboxylic acid methyl ester; SERT, serotonin transporter(s); MPP^+, 1-methyl-4-phenylpyridinium; HPLC-ECD, high-pressure liquid chromatography with electrochemical detection; ANOVA, analysis of variance; MeNER, (S,S)-2-(α-(2-methoxyphenoxy)benzyl)morpholine; n., nucleus; SCH23390, R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; GR125743, N-[4-methoxy-3-(4-methyl piperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide; MK-801, 5H-dibenzo[a,d]cyclohepten-5,10-imine (dizocilpine maleate); [125I]HEAT, [125I]iodo-2-[β-(4-hydroxyphenyl)-ethylaminomethyl]tetralone; [3H]N-methylspiperone.

are available for this disease. Despite the widespread clinical use of modafinil, the mechanisms underlying its therapeutic efficacy are not well understood (for review, see Ballon and Feifel, 2006).

Initial studies in animals demonstrated that stimulant effects of modafinil are distinct from those of amphetamine and may not involve dopamine (DA) systems in the brain (Duteil et al., 1990; Simon et al., 1995). The reported nondopaminergic effects of modafinil include activation of $\alpha 1$ adrenergic receptors (Duteil et al., 1990), enhancement of serotonin (5-HT) function (Ferraro et al., 2000), inhibition of GABA release (Ferraro et al., 1997, 1998), and stimulation of glutamate and histamine release (Ferraro et al., 1999; Ishizuka et al., 2003). With regard to the treatment of cocaine dependence, modafinil has been described as a glutamate enhancer (Dackis and O'Brien, 2003). In contrast, substantial evidence indicates that modafinil may exert its effects via presynaptic dopaminergic mechanisms (Nishino et al., 1998; Minzenberg and Carter, 2008). Mignot et al. (1994) reported in 1994 that modafinil inhibits DA transporter (DAT) binding, with an IC_{50} value of 3.2 μM (Mignot et al., 1994), whereas Madras et al. (2006) showed recently that modafinil occupies DAT and norepinephrine (NE) transporters (NET) in living primate brain. Consistent with these data, modafinil administration increases extracellular levels of DA in brain as measured by in vivo microdialysis (de Saint Hilaire et al., 2001; Wisor et al., 2001; Murillo-Rodríguez et al., 2007), and wake-promoting actions are absent in DAT-knockout mice (Wisor et al., 2001).

Based on available evidence, it seems that modafinil interacts with multiple molecular targets in the brain, including DAT proteins. Nonetheless, there are fundamental unresolved issues regarding the pharmacology of modafinil. For example, few investigations have screened the activity of modafinil at various receptors and transporters (but see Mignot et al., 1994), and no studies to our knowledge have attempted to correlate in vivo neurochemical effects of modafinil with ongoing behaviors. In the present study, we addressed these issues by first examining the activity of modafinil at a range of receptors and transporters (i.e., receptorome screen) (Armbruster and Roth, 2005). Our results identified DAT as the principal binding site for modafinil. The interaction of modafinil with DAT sites was characterized in vitro, and the effects of modafinil administration were compared with those of the indirect DA agonists GBR12909 and (+)-methamphetamine (METH). The findings show that modafinil interacts with DAT proteins as an uptake blocker, and this action is involved with stimulant properties of the drug. Importantly, modafinil pretreatment reduced behavioral and neurochemical effects of METH, supporting the drug's potential utility as a pharmacotherapy for METH dependence.

Materials and Methods

Animals and Surgical Procedures. Male Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) weighing 300 to 400 g were double-housed with food and water freely available. Rats were maintained in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and procedures were carried out in accordance with the Intramural Research Program, Animal Care and Use Committee of the National Institute on Drug Abuse (Baltimore, MD). For in vivo microdialysis studies, rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.), and then jugular catheters and intracerebral guide cannulae were implanted (Baumann et al., 2002). Guide cannulae were aimed at the n. accumbens according to coordinates ML, -1.7 mm and AP, $+1.6$ mm relative to bregma, and DV, -6.0 mm relative to dura. Rats were single-housed postoperatively and allowed at least 1 week to recover.

In Vitro Receptor and Transporter Assays. Binding and functional assays were carried out in transfected cells expressing cloned human G protein-coupled receptors or monoamine transporters, using resources of the National Institute of Mental Health Psychoactive Drug Screening Program (http://pdsp.med.unc.edu/pdspw/clones.php). Multiple receptor subtypes for DA, NE, 5-HT, GABA, glutamate, histamine, and opioids were investigated, and details of assay conditions have been published previously (Roth et al., 2002). Binding assays for monoamine transporters were also carried out in rat brain membranes; the cocaine analog [^{125}I]RTI-55 was used to label DAT and 5-HT transporters (SERT), whereas [^{3}H]nisoxetine was used to label NET. The initial screening of modafinil at receptors and transporters was performed in quadruplicate at a 10 μM concentration. In instances where modafinil displayed greater than 50% inhibition of binding, K_i values were determined using six to 10 concentrations of the drug. Uptake and release assays for DAT, NET, and SERT were carried out in rat brain synaptosomes using published methods (Rothman et al., 2003). For uptake inhibition assays, [^{3}H]DA, [^{3}H]NE, and [^{3}H]5-HT were used to assess transport activity at DAT, NET, and SERT, respectively. In release assays, [^{3}H]1-methyl-4-phenylpyridinium ([^{3}H]MPP$^+$) was used as a radiolabeled substrate for DAT and NET, whereas [^{3}H]5-HT was used as a substrate for SERT. K_i values for receptor binding, IC_{50} values for uptake inhibition, and K_e values (i.e., apparent dissociation constant of an antagonist) for METH-induced release were calculated using GraphPad Prism version 4.0 (GraphPad Software Inc., San Diego, CA).

In Vivo Microdialysis and Motor Activity. On the evening before microdialysis testing, rats were brought into the laboratory. Extension tubes were connected to catheters, and microdialysis probes (2 × 0.5 mm, CMA/12; CMA/Microdialysis, Solna, Sweden) were inserted into guide cannulae. Each rat was attached to a tether and placed into a Plexiglas arena equipped with photobeams that allowed movements to be quantified (TruScan; Coulborn Instruments, Allentown, PA). Probes were perfused with Ringers' solution at 0.6 μl/min overnight. The next morning, dialysate samples were collected at 20-min intervals and then assayed for DA and 5-HT by microbore high-pressure liquid chromatography with electrochemical detection (HPLC-ECD) (Baumann et al., 2008). After collection of three baseline samples, drug or vehicle treatments were administered intravenously through jugular catheters. Motor activity was monitored during the dialysis sampling; ambulation (i.e., forward locomotion) and stereotypy (i.e., repetitive movements) were quantified separately in 20-min bins. Raw locomotor and neurochemical data were evaluated using two-factor (treatment × time) analysis of variance (ANOVA). When significant main effects of treatment were noted, one-factor ANOVAs were run at each time point and Newman-Keuls test was used to identify differences between group means. Relationships between motor parameters and dialysate DA were determined by Pearson correlation coefficients (r). The ANOVA analyses, Pearson coefficients, and linear regressions (m) were calculated using GraphPad Prism version 4.0. $P < 0.05$ was the minimum criterion for statistical significance.

Chemicals, Reagents, and Drug Treatments. Chemicals and reagents used for in vitro assays and microdialysis were purchased from Sigma-Aldrich (St. Louis, MO), except for monochloroacetic acid, which was obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). Radiolabeled ligands were obtained from PerkinElmer Life and Analytical Sciences (Boston, MA). Alkamuls EL620 was obtained from Rhodia, Inc. (Cranbury, NY). Modafinil and GBR12909 HCl were synthesized by Dr. T. E. Prisinzano (Department of Medicinal Chemistry, The University of Kansas, Lawrence,

TABLE 1

Effects of modafinil on radioligand binding in transfected cells

Details of radioligand binding methods can be accessed via NIMH-PDSP (http://pdsp.med.unc.edu/pdspw/clones.php).

Cloned Receptor Site	Radiolabeled Ligand	K_i
		nM
DA D1	[³H]SCH23390	>10,000
DA D2	[³H]NMSP	>10,000
DA D3	[³H]NMSP	>10,000
DA D4	[³H]NMSP	>10,000
DAT	[³H]GBR12935	2600
5-HT 1A	[³H]8-Hydroxy-2-dipropylaminotetralin	>10,000
5-HT 1B	[³H]GR125743	>10,000
5-HT 2A	[³H]Ketanserin	>10,000
5-HT 2C	[³H]Mesulergine	>10,000
SERT	[³H]Citalopram	>10,000
α1A	[¹²⁵I]HEAT	>10,000
α1B	[¹²⁵I]HEAT	>10,000
α2A	[³H]Clonidine	>10,000
α2B	[³H]Clonidine	>10,000
α2C	[³H]Clonidine	>10,000
NET	[³H]Nisoxetine	>10,000
GABA$_A$, BZP	[³H]Muscimol	>10,000
Glutamate, NMDA	[³H]MK-801	>10,000
Histamine 1	[³H]Pyrilamine	>10,000
Histamine 2	[³H]Tiotidine	>10,000
Histamine 3	[³H]α-Methylhistamine	>10,000
Histamine 4	[³H]Histamine	>10,000

KS), whereas METH was provided by the Drug Supply Program, National Institute on Drug Abuse. GBR12909 and METH were dissolved in saline and administered in a volume of 1 ml/kg. Modafinil was diluted in equal parts ethanol and Alkamuls EL620, with sonication and gentle warming. Once dissolved, the modafinil solution was mixed with an equal volume of saline to yield final concentrations of 10 or 30 mg/ml. Modafinil was administered in a volume of 2 ml/kg, yielding a final administered dose of 20 or 60 mg/kg.
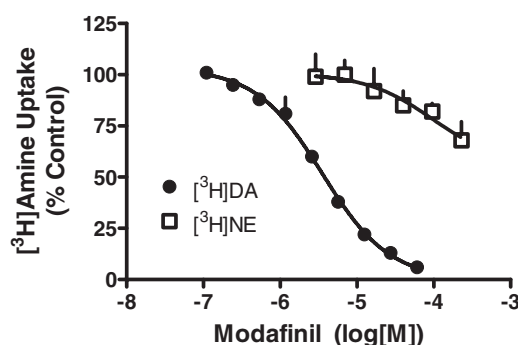
Results

In Vitro Receptor and Transporter Assays. Results from the binding screen are summarized in Table 1. When tested at a 10 μM concentration, modafinil inhibited binding of [³H]GBR12935 to human DAT, but the drug was inactive at all other sites. Subsequent analysis showed that modafinil displayed a K_i value of 2.6 μM at DAT. Given that DAT was the sole target of modafinil binding in cells, we examined the interaction of modafinil with DAT and other transporters in rat brain tissue. Table 2 demonstrates that modafinil inhibited DAT binding of the cocaine analog [¹²⁵I]RTI-55, with a K_i value of 4.8 μM but was much less potent at SERT and NET. Binding and uptake data for cocaine and GBR12909 are

Fig. 1. Effects of modafinil on inhibition of [³H]amine uptake. Various concentrations of modafinil were incubated with [³H]DA or [³H]NE in rat brain synaptosomes as described under *Materials and Methods*. Values are mean ± S.D. expressed as percentage of control uptake determined from three separate experiments.

shown for comparison. Modafinil inhibited the uptake of [³H]DA much more potently than that of [³H]5-HT and [³H]NE. For example, Fig. 1 illustrates that modafinil inhibited [³H]DA uptake, with an IC$_{50}$ value of 4.0 μM, but it was essentially inactive at [³H]NE uptake. Modafinil was inactive in transporter release assays (data not shown), suggesting the drug is a pure DA uptake inhibitor. A prediction of this hypothesis is that modafinil should antagonize METH-induced release of radiolabeled DAT substrates such as [³H]MPP⁺. In agreement with this idea, Fig. 2 shows that modafinil (10 μM) shifted the METH dose-response curve to the right for DAT-mediated release but had no effect on SERT-mediated release. The apparent dissociation constant of modafinil for antagonism of METH-induced release of [³H]MPP⁺ (i.e., K_e) was 4.3 μM, a value nearly equal to the K_i and IC$_{50}$ values determined in DAT binding and uptake inhibition assays.

In Vivo Microdialysis and Motor Activity. Given the evidence that modafinil interacts with DAT, we tested effects of the drug on motor activity and neurochemistry in rats undergoing in vivo microdialysis. Figure 3 demonstrates that intravenous modafinil produced dose-related hyperactivity characterized by increases in ambulation ($F[2,135] = 42.73$; $P < 0.0001$) and stereotypy ($F[2,135] = 91.83$; $P < 0.0001$). In the same rats, modafinil significantly elevated dialysate DA ($F[2,135] = 31.49$; $P < 0.0001$), but not 5-HT, as depicted in Fig. 4. We wanted to compare in vivo effects of modafinil with those of established DA uptake inhibitors (e.g., GBR12909) and releasers (e.g., METH). To this end, doses of intravenous GBR12909 and METH were selected to elicit the same degree of motor stimulation caused by doses of intravenous modafi-

TABLE 2

Effects of modafinil on transporter binding and uptake inhibition in rat brain

Transporter binding and uptake assays were conducted as described under *Materials and Methods*. Each value represents mean ± S.D. for three separate experiments.
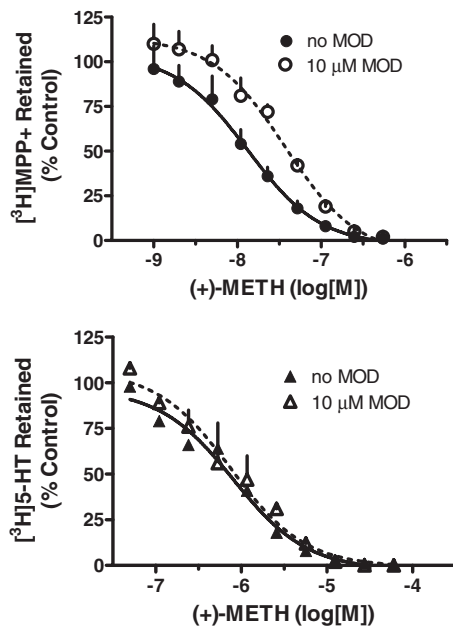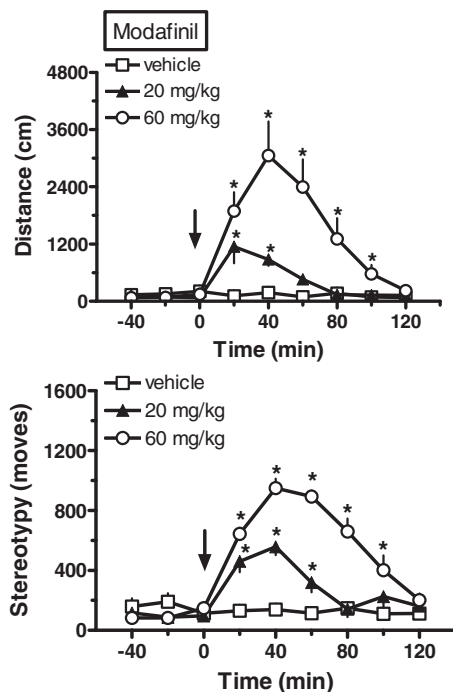
	Radioligand Binding Data			Uptake Inhibition Data		
	DAT K_I	SERT K_i	NET K_i	DAT IC$_{50}$	SERT IC$_{50}$	NET IC$_{50}$
			nM			
Modafinil	4800 ± 480	46,620 ± 8790	>92,000			
GBR12909[a]	12.0 ± 1.9	105 ± 11	497 ± 17			
Cocaine[a]	187 ± 19	172 ± 15	3210 ± 149			
Modafinil				4043 ± 385	>50,000	136,000 ± 13,900
GBR12909[b]				4.3 ± 0.3	73 ± 2	79 ± 5
Cocaine[b]				487 ± 25	304 ± 10	779 ± 30

[a] Binding data are reported in Rothman et al. (2008).
[b] Uptake inhibition data are reported in Rothman et al. (2001).
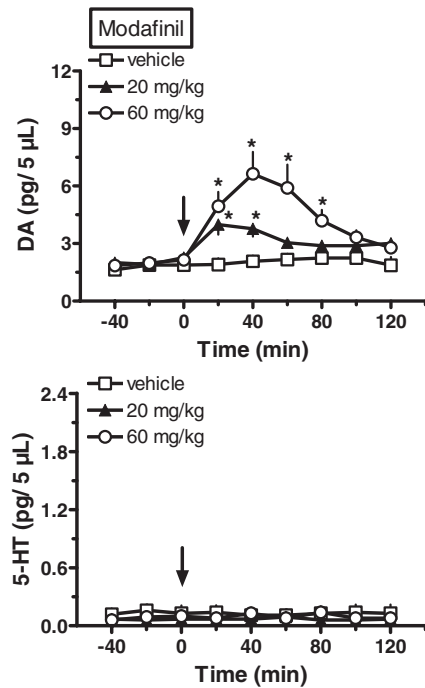
Fig. 2. Effects of modafinil on METH-induced release of [^{3}H]MPP$^+$ (top) and [^{3}H]5-HT (bottom). Various concentrations of METH were added to synaptosomes preloaded with radiolabeled substrate for DAT ([^{3}H]MPP$^+$) or SERT ([^{3}H]5-HT). Release assays were conducted in the presence or absence of 10 μM modafinil. Values are mean $\pm$ S.D. expressed as percentage of control tritium retained determined from three separate experiments.
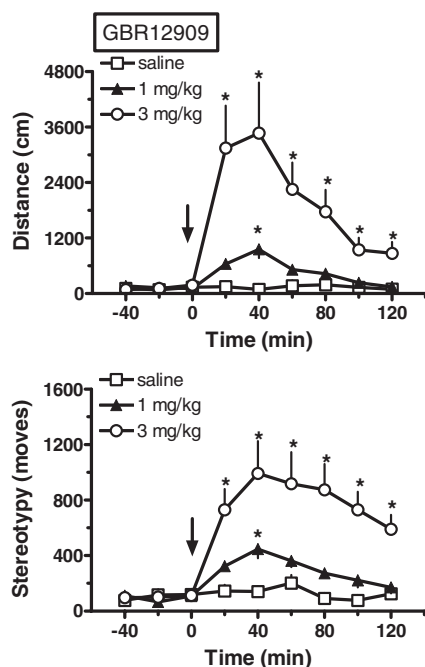

Fig. 4. Effects of modafinil on extracellular DA (top) and 5-HT (bottom) in rats undergoing microdialysis in the n. accumbens. Rats received intravenous modafinil or vehicle at time 0. Dialysate samples were collected at 20-min intervals and assayed for DA and 5-HT via HPLC-ECD. Values are mean $\pm$ S.E.M. expressed as picograms/5-μl sample for $n = 6$ rats/group. Asterisks represent significant effects compared with vehicle control at particular time points (Newman-Keuls, $P < 0.05$).

Fig. 3. Effects of modafinil on ambulation (top) and stereotypy (bottom) in rats undergoing microdialysis in the nucleus accumbens. Rats received intravenous modafinil or vehicle at time 0. Ambulation and stereotypy were measured in 20-min bins. Ambulation is expressed as distance traveled in centimeters, whereas stereotypy is expressed as number of moves. Values are mean $\pm$ S.E.M. for $n = 6$ rats/group. Asterisks represent significant effects compared with vehicle control at particular time points (Newman-Keuls, $P < 0.05$).

nil. Figure 5 shows that GBR12909 produced dose-related increases in ambulation ($F[2,135] = 41.05$; $P < 0.0001$) and stereotypy ($F[2,135] = 60.68$; $P < 0.0001$). Similar to modafi-

nil, GBR12909 elevated dialysate DA ($F[2,135] = 79.52$; $P < 0.0001$) without altering 5-HT, as illustrated in Fig. 6. It is noteworthy that increases in dialysate DA evoked by GBR12909 were much greater than those evoked by modafinil at equivalent motor stimulant doses (compare Figs. 4 and 6). Figure 7 demonstrates that intravenous METH produced dose-related increases in ambulation ($F[2,135] = 82.72$; $P < 0.0001$) and stereotypy ($F[2,135] = 99.04$; $P < 0.0001$). In contrast to modafinil and GBR12909, METH caused significant elevations in both dialysate DA ($F[2,35] = 93.85$; $P < 0.0001$) and 5-HT ($F[2,135] = 31.09$; $P < 0.0001$), as shown in Fig. 8.

Correlations between Motor Activity and DA. Because motor and neurochemical endpoints were determined concurrently for the dose-effect comparisons (Figs. 3–8), it was possible to use raw data from individual rats to construct correlation plots. To determine correlations, data from the first 60 min after injection were included: each rat provided three data points for ambulation (centimeters traveled) versus DA (picograms) and three data points for stereotypy (number of moves) versus DA (picograms). In this manner, the total number of points per plot was 54 (i.e., three treatments per drug, three time points; $n = 6$ rats/group). Figure 9 demonstrates that modafinil-induced increases in ambulation and stereotypy were positively correlated with dialysate DA. For both locomotor measures, correlations were highly significant ($P < 0.0001$). The slopes of the regression lines for ambulation versus DA and stereotypy versus DA were m = 330.1 cm/pg and m = 109.2 moves/pg, respectively. These high slope values indicate that modafinil evokes marked
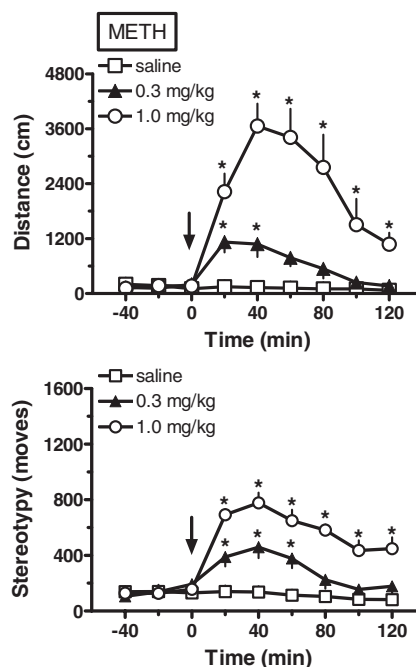
Fig. 5. Effects of the DA uptake inhibitor GBR12909 on ambulation (top) and stereotypy (bottom) in rats undergoing microdialysis in the n. accumbens. Rats received intravenous GBR12909 or saline at time 0. Ambulation and stereotypy were measured in 20-min bins. Ambulation is expressed as distance traveled in centimeters, whereas stereotypy is expressed as number of moves. Values are mean ± S.E.M. for $n = 6$ rats/group. Asterisks represent significant effects compared with saline control at particular time points (Newman-Keuls, $P < 0.05$).
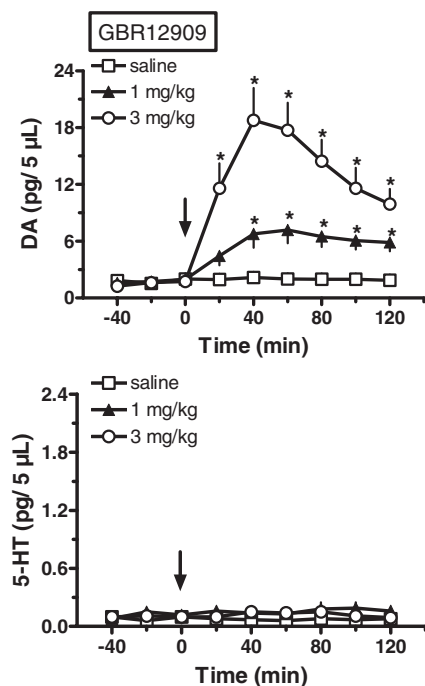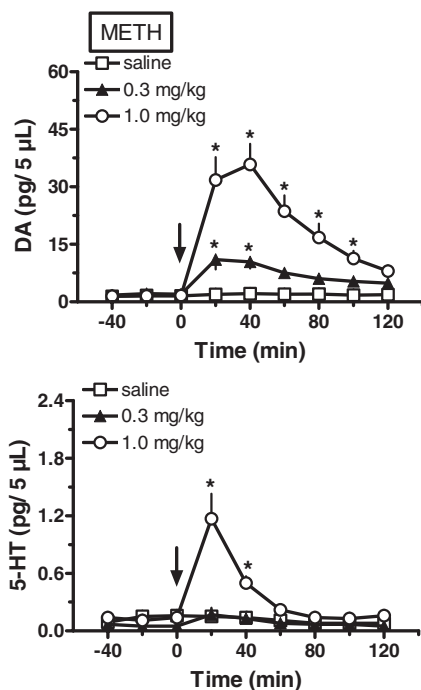

Fig. 6. Effects of GBR12909 on extracellular DA (top) and 5-HT (bottom) in rats undergoing microdialysis in the n. accumbens. Rats received intravenous GBR12909 or saline at time 0. Dialysate samples were collected at 20-min intervals and assayed for DA and 5-HT via HPLC-ECD. Values are mean ± S.E.M. expressed as picograms/5-μl sample for $n = 6$ rats/group. Asterisks represent significant effects compared with saline control at particular time points (Newman-Keuls, $P < 0.05$).


Fig. 7. Effects of the releasing agent METH on ambulation (top) and stereotypy (bottom) in rats undergoing microdialysis in the n. accumbens. Rats received i.v. METH or saline at time 0. Ambulation and stereotypy were measured in 20-min bins. Ambulation is expressed as distance traveled in centimeters, whereas stereotypy is expressed as number of moves. Values are mean ± S.E.M. for $n = 6$ rats/group. Asterisks represent significant effects compared with saline control at particular time points (Newman-Keuls, $P < 0.05$).

hyperactivity for every picogram per microliter increase in extracellular DA. The data in Fig. 10 demonstrate that motor activity produced by GBR12909 was also positively correlated with dialysate DA levels. Compared with modafinil, slopes of the GBR12909 regression lines for ambulation versus DA (m = 169) and stereotypy versus DA (m = 53.7) were much lower. Figure 11 demonstrates that METH-induced stimulation of motor activity was positively correlated with DA, and slopes of the METH regression lines for ambulation versus DA (m = 89.8) and stereotypy versus DA (m = 20.9) were less than those of GBR12909.

Effects of Modafinil on METH-Induced Responses. In the final experiment, we examined the effect of modafinil pretreatment on METH-induced locomotor and dialysate DA responses. We postulated that occupancy of DAT sites by modafinil might antagonize the motor and neurochemical effects of METH. For this experiment, modafinil was administered 40 min before intravenous METH to ensure occupancy of DAT sites. Furthermore, low doses of modafinil (20 mg/kg) and METH (0.3 mg/kg) were chosen to allow for the detection of enhanced or blunted effects of the drug combination. As depicted in Fig. 12, modafinil pretreatment alone stimulated ambulation ($F[1,70] = 15.52$; $P < 0.001$), and modafinil altered the amount of locomotion produced by subsequent METH injection ($F[1,84] = 7.21$; $P < 0.01$). Post hoc analysis (Newman-Keuls, $P < 0.05$) revealed that METH-induced ambulation was blunted in modafinil-pretreated rats, relative to saline-pretreated rats, at 20 and 40 min after METH injection. Ambulation produced by modafinil plus METH was not significantly different from modafinil plus saline at any time point. Figure 13 shows that modafinil pretreatment increased stereotypy ($F[1,70] = 18.62$; $P <$

Fig. 8. Effects of METH on extracellular DA (top) and 5-HT (bottom) in rats undergoing microdialysis in the n. accumbens. Rats received intravenous METH or saline at time 0. Dialysate samples were collected at 20-min intervals and assayed for DA and 5-HT via HPLC-ECD. Values are mean $\pm$ S.E.M. expressed as picograms/5-μl sample for $n = 6$ rats/group. Asterisks represent significant effects compared with saline control at particular time points (Newman-Keuls, $P < 0.05$).
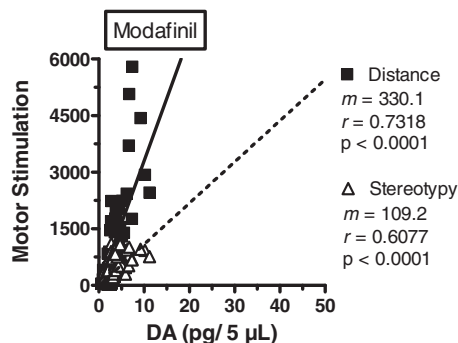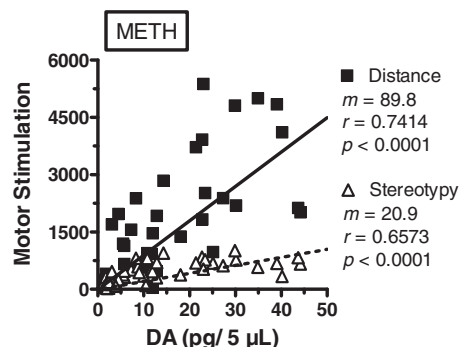

Fig. 9. Correlations between motor stimulation and dialysate DA responses produced by intravenous modafinil. Raw data from the first 60 min after injection of modafinil (20 and 60 mg/kg i.v.) and vehicle were used to construct correlation plots (see Figs. 3 and 4). Fifty-four data points contributed to correlations for ambulation (centimeters) versus DA (picograms) and stereotypy (number of moves) versus DA (picograms). Pearson correlation coefficients (r), slopes of the best-fit linear regression (m), and P values for statistical significance are given.

0.001) but had no significant effect on METH-induced stereotypy ($F[1,84] = 0.65; P < 0.4221$ N.S.). The stereotypy results suggest the ability of modafinil to reduce METH-induced ambulation is not secondary to changes in repetitive movements after the drug combination. The data in Fig. 14 demonstrate that modafinil pretreatment alone caused a modest 2-fold elevation in dialysate DA ($F[1,70] = 6.42; P < 0.01$), and modafinil altered METH-induced DA release ($F[1,84] = 7.34; P < 0.01$). Specifically, METH-induced DA release was blunted in modafinil-pretreated rats at 20 and 40 min after METH injection. METH increased dialysate DA levels in
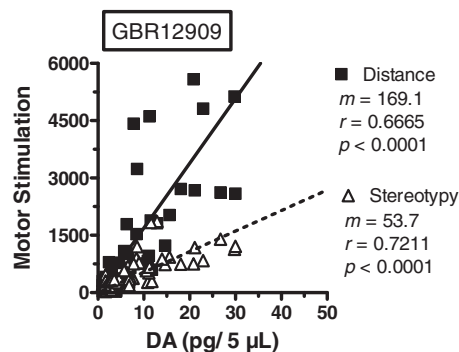
Fig. 10. Correlations between motor stimulation and dialysate DA responses produced by intravenous GBR12909. Raw data from the first 60 min after injection of GBR12909 (1 and 3 mg/kg i.v.) and saline were used to construct correlation plots (see Figs. 5 and 6). Fifty-four data points contributed to correlations for ambulation (centimeters) versus DA (picograms) and stereotypy (number of moves) versus DA (picograms). Pearson correlation coefficients (r), slopes of the best-fit linear regression (m), and P values for statistical significance are given.


Fig. 11. Correlations between motor stimulation and dialysate DA responses produced by intravenous METH. Raw data from the first 60 min after injection of METH (0.3 and 1.0 mg/kg i.v.) and saline were used to construct correlation plots (see Figs. 7 and 8). Fifty-four data points contributed to correlations for ambulation (centimeters) versus DA (picograms) and stereotypy (number of moves) versus DA (picograms). Pearson correlation coefficients (r), slopes of the best-fit linear regression (m), and P values for statistical significance are given.

modafinil-pretreated rats above the levels measured in modafinil plus saline rats.

Discussion

The major purpose of this study was to characterize the mechanism of action of modafinil, a drug often described as a wake-promoting agent with nondopaminergic actions (Ballon and Feifel, 2006). As a starting point, we screened the activity of modafinil at cloned human receptors and transporters using resources of the National Institute of Mental Health Psychoactive Drug Screening Program (http://pdsp.med.unc.edu/pdspw/clones.php) (Armbruster and Roth, 2005). The only binding site identified from the receptorome screen was DAT, where modafinil displayed a K_i value of 2.6 μM. Modafinil had no measurable affinity at monoamine receptors, suggesting that effects of the drug mediated by α1 and D2 sites (Duteil et al., 1990; Korotkova et al., 2007) might be indirect via increases in synaptic catecholamines. Experiments in rat brain tissue confirmed that modafinil inhibits DAT binding and [3H]DA uptake, with K_i and IC_{50} values of 4.0 and 5.0 μM, respectively. Two lines of evidence indicate that modafinil is a DAT inhibitor rather than a substrate-
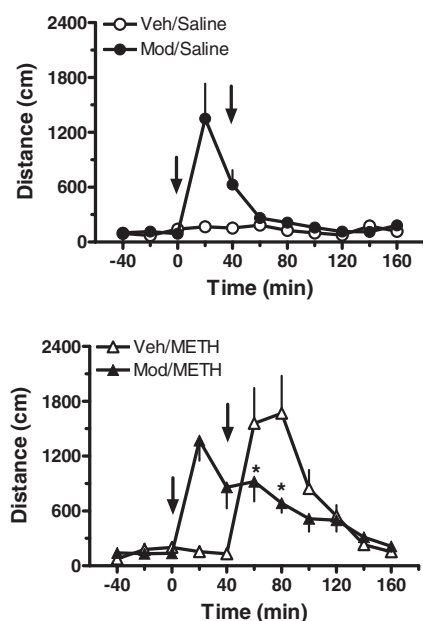
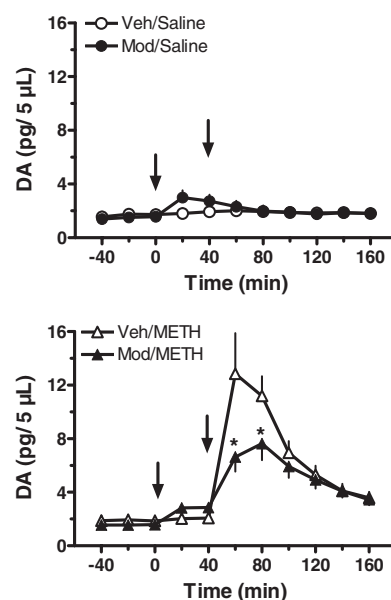**Fig. 12.** Effects of modafinil pretreatment on ambulation produced by intravenous saline (top) or METH (bottom) in rats undergoing microdialysis in n. accumbens. Pretreatment with intravenous vehicle or modafinil (20 mg/kg) was administered at time 0, followed by intravenous saline or METH (0.3 mg/kg) given 40 min later. Ambulation was measured in 20-min bins. Data are mean ± S.E.M. expressed as centimeters traveled for $n = 8$ rats/group. Asterisks represent significance with respect to vehicle plus METH treatment group at particular time points (Newman-Keuls, $P < 0.05$).

Fig. 13. Effects of modafinil pretreatment on stereotypy produced by intravenous saline (top) or METH (bottom) in rats undergoing microdialysis in n. accumbens. Pretreatment with intravenous vehicle or modafinil (20 mg/kg) was administered at time 0, followed by intravenous saline or METH (0.3 mg/kg) given 40 min later. Stereotypy was measured in 20-min bins. Data are mean ± S.E.M. expressed as number of moves for $n = 8$ rats/group.

Fig. 14. Effects of modafinil pretreatment on the dialysate DA response produced by intravenous saline (top) or METH (bottom) in rats undergoing microdialysis in the n. accumbens. Pretreatment with intravenous vehicle or modafinil (20 mg/kg) was administered at time 0, followed by intravenous saline or METH (0.3 mg/kg) given 40 min later. Dialysate samples were collected every 20 min and assayed for DA by HPLC-ECD. Data are mean ± S.E.M. expressed as picograms/5-μl sample for $n = 8$ rats/group. Asterisks represent significance with respect to vehicle plus METH treatment group at particular time points (Newman-Keuls, $P < 0.05$).

ing that 1 μM modafinil inhibits amphetamine-induced release of [3H]DA from rat striatal slices (Dopheide et al., 2007). These same investigators observed that 100 μM modafinil can release [3H]DA in a nomifensine-reversible manner, but pharmacokinetic studies are needed to determine whether brain concentrations of the drug reach this high level after clinically relevant doses.

Our in vitro data are consistent with those of Madras et al. (2006) who examined effects of modafinil in human embryonic kidney cells expressing cloned human DAT, NET, and SERT. They showed that modafinil inhibited [3H]DA uptake, with an IC_{50} value of 6.4 μM, but it had much weaker effects on [3H]NE and [3H]5-HT uptake. The negligible potency of modafinil at NET and SERT predicts the drug should not occupy these sites in vivo. Surprisingly, Madras et al. (2006) found that modafinil inhibited binding of the NET-selective positron emission tomography ligand [11C](S,S)-2-(α-(2-methoxyphenoxy)benzyl)morpholine (MeNER), in monkey thalamus. One interpretation of these data is that modafinil binds to NET sites in vivo but not in vitro. An alternative explanation is that modafinil decreases [11C]MeNER binding secondary to DAT inhibition and elevation of extracellular DA. It seems probable that in vivo binding of [11C]MeNER is sensitive to endogenous NET substrates (Seneca et al., 2006), and DA displays equal potency with NE in this regard (Rothman et al., 2001).

Having established that modafinil is a DAT inhibitor, we evaluated locomotor effects of the drug in rats undergoing microdialysis in the n. accumbens. The n. accumbens is an important limbic-motor interface that mediates behavioral effects of stimulant drugs (Swerdlow et al., 1986; Pennartz et al., 1994). Modafinil produced dose-related increases in am-

type releaser: 1) modafinil did not display substrate activity when tested in release assays, and 2) modafinil shifted the METH dose-effect curve to the right for DAT-mediated release. The ability of modafinil to antagonize METH-induced release of DAT substrates agrees with recent findings show-

bulation and stereotypy that were associated with parallel elevations in extracellular DA. Relatively high doses of modafinil were required to stimulate in vivo effects, and this probably reflects the low potency of the drug at DAT. Our microdialysis results are the first to demonstrate that systemically administered modafinil elevates extracellular DA in the n. accumbens of freely moving rats, a finding consistent with reports showing that modafinil increases dialysate DA in other brain areas of conscious rats and dogs (de Saint Hilaire et al., 2001; Wisor et al., 2001). Administration of GBR12909 or METH also increased motor activity and dialysate DA, but effects of these drugs were quantitatively different from those of modafinil. Specifically, modafinil produced a much smaller rise in extracellular DA compared with the effects of equivalent motor stimulant doses of GBR12909 or METH. This observation is reminiscent of the work of Ferraro et al. (1997) who reported that elevations in dialysate DA produced by modafinil are less than those produced by GBR12909, nomifensine, or amphetamine. Importantly, the Ferraro study involved administration of single drug doses to halothane-anesthetized rats, precluding any assessment of dose-response relationships or the role of DA in mediating behaviors.

Given the modest elevations in extracellular DA produced by modafinil, it might be assumed that dopaminergic effects are not important for motor activation. However, modafinil-induced increases in ambulation and stereotypy were positively correlated with dialysate DA in the accumbens ($P <$ 0.0001; Fig. 9). To our knowledge, the correlation data presented here are the only published data where neurochemical effects of modafinil are related to ongoing behaviors in the same subjects. Like the modafinil results, the effects of GBR12909 and METH on motor activity were strongly correlated with dialysate DA. Previous studies have reported significant positive correlations between dialysate DA and locomotor stimulation induced by amphetamine (Sharp et al., 1987), cocaine (Chen and Reith, 1994), and 3,4-methylenedioxymethamphetamine (Baumann et al., 2008). Taken together, the data suggest that hyperactivity produced by modafinil is at least partially dependent upon increases in mesolimbic DA, similar to effects of other stimulants. An important finding from the correlations is that modafinil displays steep slope values for ambulation versus DA and stereotypy versus DA. Stated more simply, modafinil is able to elicit large increases in motor activation per unit rise in dialysate DA. The reason for the high activity/DA ratio of modafinil is unclear but could be related to nondopaminergic effects that augment motor stimulation, including decreases in extracellular GABA (Ferraro et al., 1997, 1998) or increases in extracellular glutamate and histamine (Ferraro et al., 1999; Ishizuka et al., 2003).

A legitimate question is whether the present findings are relevant to the wake-promoting properties of modafinil, especially because we measured motor activity rather than wakefulness per se. It is notable that modafinil and other drugs were administered during the light phase in our study, a time when vehicle-treated rats are asleep or inactive. Therefore, the locomotor effects of modafinil reported here required rats to either wake up from sleep or be aroused from inactivity. There is a growing consensus that central DA systems are involved in maintaining arousal, and most wake-promoting medications, including modafinil, display the com-

mon feature of increasing extracellular DA (Fig. 4) (Nishino et al., 1998). A recent study showed that an intracerebroventricular injection of modafinil increases wakefulness and decreases slow wave sleep for several hours (Murillo-Rodríguez et al., 2007). The same intracerebroventricular treatment caused elevations of extracellular DA in the accumbens that resemble those shown here after intravenous modafinil administration.

In the final experiment, acute effects of METH were compared in vehicle- and modafinil-pretreated rats. Given that modafinil antagonizes METH-induced release of DAT substrates in vitro, we surmised that similar effects might be observed in vivo. Indeed, modafinil pretreatment significantly attenuated the effects of METH on ambulation and DA release, while having little effect on METH-induced stereotypy. The microdialysis findings with modafinil are much like our previous findings with GBR12909 and its analogs—pretreatment with these drugs substantially reduces DA release produced by intravenous amphetamine or METH (Baumann et al., 1994, 2002). Evidence indicates that GBR12909 and related compounds can prevent DA-releasing effects of METH by persistent occupation of DAT sites or internalization of DAT proteins (Kunko et al., 1997; Baumann et al., 2002). Arguably the most intriguing finding reported here is that modafinil pretreatment diminishes METH-induced ambulation. It seems paradoxical that modafinil can stimulate motor activity yet also inhibit ambulatory effects of METH. The molecular underpinnings of this action are not known, but the ability of modafinil to decrease METH-induced DA release could be involved. Clearly, more investigation is warranted to examine the interactions between modafinil and METH.

As noted under Introduction, modafinil pretreatment can reduce cocaine self-administration (Hart et al., 2008) and subjective effects (Dackis et al., 2003; Malcolm et al., 2006) in human drug users. Furthermore, modafinil has been tested as a medication for cocaine dependence in a double-blind placebo-controlled trial, and the results are encouraging (Dackis et al., 2005). The pharmacological basis for the efficacy of modafinil in treating cocaine dependence is not known, but given the data reported here and previously (de Saint Hilaire et al., 2001; Wisor et al., 2001; Madras et al., 2006; Murillo-Rodríguez et al., 2007), we speculate that inhibition of DAT function is a critical factor. Several selective DA uptake inhibitors, including analogs of GBR12909 and benztropine, are being developed as agonist medications for cocaine dependence (for review, see Rothman et al., 2008). Molecular investigations reveal that GBR12909 and benztropine bind to DAT in a unique manner, distinct from the interaction of cocaine with this protein (Vaughan et al., 1999; Chen et al., 2004). Interestingly, the chemical structure of modafinil shares a diphenylmethyl moiety with these candidate medications, suggesting that modafinil might display a similar molecular mechanism at DAT.

In summary, the present findings demonstrate that modafinil interacts with DAT to block DA uptake in nervous tissue. Modafinil-induced inhibition of DA uptake increases extracellular levels of DA in rat n. accumbens, and this action is involved with stimulant effects of the drug. It seems likely that nondopaminergic mechanisms that were not identified by the receptorome screen also contribute to the pharmacology of modafinil, and such mechanisms require elucidation.

Modafinil displays properties in common with agonist medications currently being investigated for the treatment of cocaine dependence. In this regard, our results suggest that further research is warranted to explore the efficacy of modafinil as a treatment for METH dependence.

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